RNA clean-up

User manual

NucleoSpin® RNA Clean-up XS

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSpin® RNA Clean-up XS		
	10 preps	**50 preps**	**250 preps**
REF	**740903.10**	**740903.50**	**740903.250**
Clean-up Buffer RCU (Concentrate)*	1 mL	5 mL	25 mL
Wash Buffer RA3 (Concentrate)*	2 mL	7 mL	2 x 20 mL
RNase-free H_2O	5 mL	15 mL	25 mL
NucleoSpin® RNA XS Binding Columns (light blue rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	10	50	250
Collection Tubes (1.5 mL)	10	50	250
User manual	1	1	1

* For preparation of working solutions and storage conditions see section 3.

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to prepare Wash Buffer RA3 and to adjust RNA binding conditions)

Consumables

- 1.5 mL microcentrifuge tubes
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Clean-up XS** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com*.**

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

A major aspect of RNA clean-up is preventing degradation of the RNA during the clean-up procedure. The **NucleoSpin® RNA Clean-up XS** method achieves this by mixing the crude RNA extract with a binding buffer, containing chaotropic ions, and ethanol. This buffer immediately inactivates RNases (which are present in virtually all biological materials) and creates appropriate binding conditions to allow adsorption of RNA to the silica membrane. Two washing steps with a single buffer remove any impurities. Pure RNA is finally eluted at low ionic strength conditions with RNase-free water (supplied) in a volume as small as 5 µL.

The RNA clean-up procedure using **NucleoSpin® RNA Clean-up XS** kit can be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often present on general lab ware, fingerprints, and dust. To ensure RNA stability, we recommend keeping the RNA solution frozen at -20 °C for short-term or -70 °C for long-term storage.

2.2 Kit specifications

- The **NucleoSpin® RNA Clean-up XS** kit is recommended for the clean-up and concentration of prepurified RNA samples. Typical sample material covers nanogramm to microgramm amounts of prepurified RNA (e.g., phenol-purified RNA) and RNA from reaction mixtures (e.g., DNase treated samples).

- The innovative column design with a funnel shaped thrust ring and a small silica membrane area allows sample volumes of up to 300 µL and elution of RNA in as little as 5–30 µL. Thus, **highly concentrated RNA** is eluted and is ready for common downstream applications (e.g., RT-PCR). RNA enrichment of 20 x up to 50 x can be achieved (e.g., input: 300 µL sample containing crude RNA (10 ng/µL); output: 5 µL eluate containing pure RNA (510 ng/µL); enrichment of factor 51 (MACHEREY-NAGEL in-house data)).

- The **RNA recovery rate** is typically 85–95 %.

- High quality RNA (RNA Integrity Number (RIN) > 9 according to Agilent 2100 Bioanylzer assays) can be obtained from high quality RNA samples. The RIN of the processed sample is typically equal (±0.3) to the RIN of the input sample. RNA quality always depends on the sample quality, see section 6.3 for further aspects.

- The **NucleoSpin® RNA Clean-up XS** kit allows clean-up and concentration of RNA with an A_{260}/A_{280} ratio generally exceeding 1.9 (measured in TE buffer pH 7.5). Due to the **high RNA purity**, large amounts of eluates can be used as template in RT-PCR without inhibition (e. g., 8 µL of 10 µL eluates as template in a 20 µL qRT-PCR setup generating stronger signal compared to reactions

with less template in a LightCycler™ PCR with the Sigma SYBR® Green Quantitative RT-PCR Kit).

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® RNA Clean-up XS
Technology	Silica-membrane technology
Format	Mini spin columns – XS design
Sample material	< 300 µL RNA solution containing < 90 µg RNA
Fragment size	> 200 nt
Typical recovery	85–95 %
A_{260}/A_{280}	1.9–2.1
Elution volume	5–30 µL
Preparation time	Approx. 20 min/6 preps
Binding capacity	110 µg

2.3 Handling, preparation, and storage of starting materials

RNA intended to be used as sample for the **NucleoSpin® RNA Clean-up XS** procedure should be handled with the same care as any RNA sample. The stability of prepurified RNA samples (e.g., RNA isolated with phenol based protocols) depends very much on the performed procedure.

Wear gloves at all times during the preparation. Change gloves frequently.

2.4 Elution procedures

A high RNA concentration in the elution fraction is desirable for all typical downstream applications. In particular with regard to limited volumes of reaction mixes, high RNA concentration can be a crucial criterion. Due to a high default elution volume, standard kits often result in low concentrated RNA, if only small samples are processed.

Such RNA often even requires a subsequent concentration to be suitable for the desired application.

In contrast to standard kits, **NucleoSpin® RNA Clean-up XS** allows an efficient elution in a very small volume resulting in highly concentrated RNA.

Elution volumes in the range of 5–30 µL are recommended, the default volume is 10 µL.

2.5 Stability of isolated RNA

Eluted RNA should immediately be put and always kept on ice during work for optimal stability! Contamination with almost omnipresent RNases (general lab ware, fingerprints, dust) may be a risk for isolated RNA. For short-term storage freeze at -20 °C, for long-term storage freeze at -70 °C.

3 Storage conditions and preparation of working solutions

Attention: Buffers RCU contains chaotropic salt. Wear gloves and goggles!

CAUTION: Buffer RCU contains guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components should be stored at room temperature (18–25 °C) and are stable up to one year. Storage at lower temperatures may cause precipitation of salts.
- Check that 96–100 % ethanol is available as additional solution in the lab.

Before starting any **NucleoSpin® RNA Clean-up** protocol, prepare the following:

- **Clean-up Buffer RCU:** Add the indicated volume of 96–100 % ethanol to the Clean-up Buffer RCU Concentrate. See table below or bottle label for necessary volumes. Store Buffer RCU at room temperature (18–25 °C) for up to one year.
- **Wash Buffer RA3:** Add the indicated volume of 96–100 % ethanol (see table below) to Wash Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RA3 at room temperature (18–25 °C) for up to one year.

	NucleoSpin® RNA Clean-up		
	10 preps	**50 preps**	**250 preps**
REF	**740903.10**	**740903.50**	**740903.250**
Clean-up Buffer RCU (Concentrate)	1 mL Add 3 mL ethanol	5 mL Add 15 mL ethanol	25 mL Add 75 mL ethanol
Wash Buffer RA3 (Concentrate)	2 mL Add 8 mL ethanol	7 mL Add 28 mL ethanol	2 x 20 mL Add 80 mL ethanol to each bottle

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® RNA Clean-up XS** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

4.1 Risk and safety phrases

Component *Inhalt*	Hazard contents *Gefahrstoff*	Hazard symbol *Gefahrstoff-symbol*	Risk phrases *R-Sätze*	Safety phrases *S-Sätze*
RCU	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	Xn*	R 20/21/22-32-52/53	S 13-61

Risk phrases

R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
Gesundheitsschädlich beim Einatmen, Verschlucken und Berührung mit der Haut.

R 32 Contact with acids liberates very toxic gas.
Entwickelt bei Berührung mit Säure sehr giftige Gase.

R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Schädlich für Wasserorganismen, kann in Gewässern längerfristig schädliche Wirkungen haben.

Safety phrases

S 13 Keep away from food, drink and animal foodstuffs.
Von Nahrungsmitteln, Getränken und Futtermitteln fernhalten.

S 61 Avoid release to the environment. Refer to special instructions / safety data sheet.
Freisetzung in die Umwelt vermeiden. Besondere Anweisungen einholen / Sicherheitsdatenblätter zu Rate ziehen.

* Hazard labeling not neccessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

4.2 GHS classification

Only harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
RCU	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	Warning *Achtung*	302, 412, EUH031	260, 273, 301+312, 330

Hazard phrases

H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
EUH031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P 260	Do not breathe vapours. *Dampf nicht einatmen.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 301+312	IF SWALLOWED: Call a POISON CENTER or doctor /physician if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM oder Arzt anrufen.*
P 330	Rinse mouth. *Mund ausspülen.* *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM oder Arzt anrufen.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***

Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

5 Protocols

5.1 RNA clean-up and concentration of RNA

Before starting the preparation:

- Check if Buffer RCU and Buffer RA3 were prepared according to section 3.

1 Sample preparation

Provide up to **300 µL sample** containing up to 90 µg RNA – such as prepurified RNA (e.g., phenol purified) or RNA from reaction mixtures (e.g., labelling reactions) – in a microcentrifuge tube (not provided).

For appropriate sample amounts see section 2.3.

Note: Fill up RNA samples smaller than 100 µL with RNase-free water to 100 µL. RNA samples from 100–200 µL should be filled up with RNase-free water to 200 µL.

2 Adjust RNA binding conditions

Add **one volume of Buffer RCU** to the sample (e.g., 100 µL RCU to 100 µL sample) and **mix 2 x 5 s**. If necessary, spin down gently (approx. 1 s at 1,000 x *g*) to clean the lid.

+ 1 vol. RCU

Mix (2 x 5 s)

3 Bind RNA

Take one NucleoSpin® RNA XS Column (light blue ring) placed in a Collection Tube for each preparation. Load up to **300 µL sample mix** to the column. Centrifuge for **30 s** at **11,000 x *g***.

For volumes exceeding 300 µL, load the sample mix in two subsequent centrifugation steps onto the column.

Load sample mix

Place the column in a new Collection Tube (2 mL).

11,000 x *g*, 30 s

Maximal loading capacity of NucleoSpin® RNA XS Columns is 600 µL. However, for maximum performance loading at most 300 µL onto the column for one centrifugation step is recommended. For larger volumes, load the sample mix in two (or more if necessary) successive centrifugation steps. Repeat the procedure if larger volumes are to be processed. For high demanding applications, the recovery rate can further be increased as follows: Centrifuge 30 s at 2,000 x g *prior to centrifugation for 30 s at 11,000 x* g.

4 Wash and dry silica membrane

1st wash — **+ 400 µL RA3**

Add **400 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column back into the Collection Tube.

11,000 x *g*, 30 s

2nd wash — **+ 200 µL RA3**

Add **200 µL Buffer RA3** to the NucleoSpin® RNA XS Column. Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL, supplied).

11,000 x *g*, 2 min

If for any reason, the liquid level in the Collection Tube has reached the NucleoSpin® RNA XS Column after centrifugation, discard flow-through and centrifuge again.

5 Elute RNA

Elute the RNA in **10 µL RNase-free H_2O**, (supplied) and centrifuge at **11,000 x *g***. for **30 s**.

+ 10 µL RNase-free H_2O

11,000 x *g*, 30 s

If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5–30 µL.

For further details on alternative elution procedures see section 2.4.

5.2 DNA digestion in crude RNA extracts and subsequent clean-up

Several commonly used RNA purification methods co-purify DNA to a considerable extent (e.g., phenol based RNA purification). This often requires a subsequent removal of contaminating DNA and clean-up of the RNA from the reaction mixture.

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase, and digested DNA) are usually required.

The MACHEREY-NAGEL rDNase Set (to be ordered separately, see ordering information), contains high quality, recombinant RNase-free DNase (rDNase) and reaction buffer. It is optimized for a highly efficient digestion in order to remove even traces of contaminating DNA.

1 Digest DNA (reaction setup)

Prepare enzyme-buffer premix: Add **1 µL rDNase** to **10 µL Reaction Buffer for rDNase**.

Add 1/10 volume of enzyme-buffer premix to the crude RNA extract (e.g., to 10 µL RNA extract add 1 µL of the premix comprising buffer and enzyme).

Gently swirl the tube in order to mix the solutions. Spin down gently (approx. 1 s at 1,000 x *g*) to collect every droplet of the solution at the bottom of the tube.

Note: Dissolve lyophilized rDNase (rDNase Set, see ordering information) in 540 µL RNase-free H_2O as described in the corresponding user manual.

2 Incubate sample

Incubate for **10 min** at **37 °C**.

3 Repurify RNA

Repurify RNA with the NucleoSpin® RNA Clean-up XS kit according to section 5.1.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/no RNA obtained	*RNase contamination* • Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use.
Poor RNA quality or yield	*Reagents not applied or restored properly* • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No ethanol has been added to Clean-up Buffer RCU. Binding of RNA to the silica membrane is only effective in the presence of ethanol. Adjust binding conditions by adding ethanol to Clean-up Buffer RCU Concentrate as described in section 3. *Kit storage* • Store kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. *Ionic strength and pH influence A_{260} absorption as well as ratio A_{260}/A_{280}* • For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also: - Manchester, K L. 1995. Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. Biotechniques 19, 208–209. - Wilfinger, W W, Mackey, K and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474–481. *Sample material* • Sample material not stored properly. Keep thawed samples on ice before addition of Buffer RCU.

Problem	Possible cause and suggestions
Contamination of RNA with genomic DNA	*Sample material already contaminated with DNA* • Digest contaminating DNA in an RNA sample according to section 5.2.
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • Do not let the flow-through touch the column outlet after the second wash using Wash Buffer RA3. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Wash Buffer RA3 completely. • Check if Wash Buffer RA3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Wash Buffer RA3. • Depending on the robustness of the used RT-PCR system, RT-PCR might be inhibited if complete eluates are used as template for RT-PCR. Use less eluate as template. *Store isolated RNA properly* • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C.
Higher RNA yield than theoretically possible	• If performing clean-up of samples containing less than approximately 300 ng RNA, subsequent quantification by A_{260} measurement may simulate yields larger than the RNA input. This may be due to absorbance of silica abrasion. In order to prevent incorrect A_{260} quantification of small RNA amounts, centrifuge the elution tube for 30 s at 8.000–11.000 x *g* and withdraw an aliquot for measurement without disturbing any sediment or use a silica abrasion insensitive RNA quantification method (e.g., RiboGreen fluorescent dye).
Unexpected A_{260}/A_{280} ratio	*Measurement not in the range of photometer detection limit* • In order to obtain a significant A_{260}/A_{280} ratio it is necessary that the initially measured A_{260} and A_{280} values are significantly above the detection limit of the photometer used. An A_{280} value close to the background noise of the photometer will cause unexpected A_{260}/A_{280} ratios.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® RNA Clean-up XS	740903.10	10 preps
	740903.50	50 preps
	740903.250	250 preps
NucleoSpin® RNA XS	740902.10	10 preps
	740902.50	50 preps
	740902.250	250 preps
NucleoSpin® RNA II	740955.20	20 preps
	740955.50	50 preps
	740955.250	250 preps
NucleoSpin® RNA L	740962.20	20 preps
NucleoSpin® RNA/Protein	740933.10	10 preps
	740933.50	50 preps
	740933.250	250 preps
NucleoSpin® TriPrep*	740966.10	10 preps
	740966.50	50 preps
	740966.250	250 preps
NucleoSpin® RNA Clean-up	740948.10	10 preps
	740948.50	50 preps
	740948.250	250 preps
NucleoSpin® miRNA	740971.10	10 preps
	740971.50	50 preps
	740971.250	250 preps
NucleoSpin® RNA Blood	740200.10	10 preps
	740200.50	50 preps
NucleoSpin® RNA Plant	740949.10	10 preps
	740949.50	50 preps
	740949.250	250 preps
NucleoSpin® FFPE RNA	740969.10	10 preps
	740969.50	50 preps
	740969.250	250 preps
NucleoSpin® RNA/DNA Buffer Set*	740944	Suitable for 100 preps
rDNase Set	740963	1 set

* DISTRIBUTION AND USE OF NUCLEOSPIN® TRIPREP and NUCLEOSPIN® RNA/DNA BUFFER SET IN THE USA IS PROHIBITED FOR PATENT REASONS.

Product	REF	Pack of
NucleoSpin® Filters	740606	50
Collection Tubes (2 mL)	740600	1000

6.3 Literature

Fleige S, Pfaffl MW.: RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med. 2006 Apr-Jun; 27(2-3):126-39. Epub 2006 Feb 15. Review.

Imbeaud S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A, Auffray C.: Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. Nucleic Acids Res. 2005 Mar 30;33(6):e56.

Miller CL, Diglisic S, Leister F, Webster M, Yolken RH.: Evaluating RNA status for RT-PCR in extracts of postmortem human brain tissue. Biotechniques. 2004 Apr; 36(4):628-33.

Schoor O, Weinschenk T, Hennenlotter J, Corvin S, Stenzl A, Rammensee HG, Stevanovic S.: Moderate degradation does not preclude microarray analysis of small amounts of RNA. Biotechniques. 2003 Dec; 35(6):1192-6, 1198-201.

6.4 Product use restriction/warranty

NucleoSpin® RNA Clean-up XS kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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